Acute and Chronic Effects of Cigarette Smoking on Exhaled Nitric Oxide

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Cigarette smoking is associated with an increased risk of respiratory tract infections, chronic airway disease, and cardiovascular diseases, all of which may be modulated by endogenous nitric oxide (NO). We have investigated whether cigarette smoking reduces the production of endogenous NO. We compared exhalations of 41 current cigarette smokers with normal lung function and 73 age-matched nonsmoking controls. Peak exhaled NO levels were measured by a modified chemiluminescence analyzer. The effects of inhaling a single cigarette in smokers were also measured. In control subjects we also measured the effects of inhalation of NO itself and carbon monoxide, both constituents of tobacco smoke. Peak exhaled NO concentrations were significantly reduced in smokers (42 ± 3.9 compared with 88 ± 2.7 parts per billion in nonsmokers, $p < 0.01$), with a significant relation between the exhaled NO and cigarette consumption ($r = -0.77$, $p < 0.001$). Smoking a single cigarette also significantly ($p < 0.02$), but transiently, reduced exhaled NO. Inhalation of carbon monoxide and NO had no effect on exhaled NO in normal subjects. Cigarette smoking decreased exhaled NO, suggesting that it may inhibit the enzyme NO synthase. Since endogenous NO is important in defending the respiratory tract against infection, in counteracting bronchoconstriction and vasoconstriction, and in inhibiting platelet aggregation, this effect may contribute to the increased risks of chronic respiratory and cardiovascular disease in cigarette smokers. Kharitonov SA, Robbins RA, Yates D, Keatings V, Barnes PJ. Acute and chronic effects of cigarette smoking on exhaled nitric oxide.

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Cigarette smoking is associated with several adverse health consequences, including an increased risk of cardiovascular diseases and respiratory tract infections (1–4). Although many factors have been implicated in the increased incidence of these problems, the mechanisms have remained poorly defined. The effect of cigarette smoke on the respiratory tract has been ascribed both to a toxic action of cigarette smoke constituents on epithelial cell function, which may interfere with clearance of particles from the lung, and to an effect on alveolar macrophages, resulting in an influx of neutrophils into the respiratory tract. The mechanism for the increased cardiovascular risk from cigarette smoke is poorly understood, but it is presumed to be due to the absorption of tobacco smoke constituents that may affect endothelial cell function in some way. We now describe a mechanism that could contribute to the increased risk of these diseases in cigarette smokers.

Endogenous nitric oxide (NO) plays a key role in cell signaling (5, 6) and in host defense (7, 8). Nitric oxide plays an important role in the respiratory tract, including the regulation of pulmonary and airway blood flow and in nonspecific defense of the respiratory tract (9). Nitric oxide is formed from L-arginine by the activity of NO synthase (NOS), of which at least three isoforms have been identified in human airways (9). Constitutive forms of NOS include an endothelial form (eNOS), which mediates endothelium-dependent vasodilator responses, and a neuronal form (nNOS) that is present in airway nerves (10) and involved in neural bronchodilatation (11). There is also an isoform induced by proinflammatory cytokines and bacterial products (iNOS), which is expressed in macrophages and airway epithelial cells (12–14). The expression of iNOS in airway epithelial cells and macrophages may be important in defense of the respiratory tract against infectious agents that are inhaled (7, 8).

Recently it has become apparent that NO itself may have an inhibitory feedback effect on NOS in a variety of cell types (15–17). Cigarette smoke contains a high concentration of NO (18), and we hypothesized that cigarette smoking may therefore reduce the formation of endogenous NO. Nitric oxide is detectable in the exhaled air of animals and normal humans (19), and the concentration of NO is increased in the exhaled air of patients with asthma and bronchiectasis (20–22). This increase is presumed to be due to induction of iNOS by cytokines released in chronic inflammation of the airways. In support of this presumption, there is immunocytochemical evidence for an increase in iNOS expression in the airway epithelium of patients with asthma (23). Interestingly, a preliminary report suggests that the concentration of exhaled NO may be reduced in a small number of cigarette smokers (21).

To explore the effect of cigarette smoking on exhaled NO, we compared the levels of NO in the exhaled air of chronic cigarette smokers with those of age-matched nonsmoking control subjects,

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TABLE 1
SUBJECT CHARACTERISTICS

	Number	Age (yr)	Sex (M/F)	Cigarettes/day	Pack-years	FEV_1 (% predicted)
Nonsmokers	73	35 ± 0.9	44/29	0	0.1 ± 0.1*	101 ± 8
Smokers	41	34 ± 1.9	17/24	15.3 ± 1.5	10.2 ± 1.3	98 ± 4

Mean ± SEM values shown.
* Includes one ex-smoker.

and we investigated the acute effect of cigarette smoking in cigarette smokers. Cigarette smoke contains carbon monoxide (CO), which might inhibit NOS activity, because NOS has a heme structure related to cytochrome P450 and is potently inhibited by CO (24). As cigarette smoke itself contains NO (18), we have also studied whether inhaled NO reduces exhaled NO in normal individuals.

METHODS

Patients. We studied 73 normal nonsmokers of either sex and 41 age-matched active cigarette smokers who were recruited from the staff or outpatients of Royal Brompton Hospital (Table). The protocol was approved by the local Ethics Committee. Subjects were excluded if they had any history of past or current respiratory disease, if they had had an upper respiratory tract infection within the last 6 wk, or if they were taking any chronic medication or antibiotics. Cigarette smokers were asked to refrain from cigarette smoking for at least 8 h before the measurement of exhaled NO. Lung function was measured by spirometry (Vitalograph, Buckingham, UK) in all subjects before measurement of exhaled NO.

Measurement of exhaled nitric oxide. Exhaled NO was measured using a modified chemiluminescence analyzer (Dasibi Environmental Corporation, Glendale, CA) sensitive to NO at 2–4000 parts per billion (ppb, vol/vol), which was adapted for on-line recording of NO concentration, as previously described (20). After we flushed the analyzer with NO-free compressed air, subjects performed a slow vital capacity maneuver over 30–45 s into wide-bore Teflon tubing, and NO was sampled continuously at a rate of 250 ml/min. Subjects wore nose clips for all measurements. Results were displayed on a chart recorder and compared with the signal generated from a calibration mixture of NO (89 ppb) in N_2 (British Oxygen Corporation, London, England). Previous analysis of the concentration traces showed that the area under the curve was highly correlated with the peak value ($r = 0.98$), and peak values were therefore used in all calculations. Three successive peak values were recorded and the mean values analyzed. Ambient-air NO levels (0–68 ppb) were found to have no effect on the values of exhaled NO.

Measurements of exhaled NO in individuals were reproducible on separate days. In 33 normal subjects the measurement was repeated on a separate day, and variation between measurements varied from 0 to 14%.

Acute smoking exposure. To determine the acute effects of cigarette smoke, 17 smoking subjects were selected who had exhaled NO concentrations of > 20 ppb. Subjects refrained from smoking for at least 8 h and, after baseline measurements of exhaled NO had been made, smoked one cigarette (Camel unfiltered; R. J. Reynolds, Winston-Salem, NC) and were encouraged to inhale deeply. Peak exhaled NO levels were measured immediately before and then 5 to 15 min after smoking.

Effect of carbon monoxide. Five normal subjects inhaled a gas mixture containing 0.29% carbon monoxide, 18% O_2, 13.7% helium, and the balance of N_2 for 5 min. Carbon dioxide in expired air was measured using a Micro Smokerlyzer (Bedfont Scientific, Upchurch, UK). Exhaled NO and CO concentrations were measured before and then at 5 and 15 min after CO inhalation.

Effect of nitric oxide. Eight normal subjects inhaled a high concentration of NO gas (9,980 ppb) for ten vital-capacity breaths over 2 min. Peak exhaled NO was measured before and then at 5 and 15 min after the last inhalation.

Statistical analysis. All data are expressed as means ± standard error. Treatment groups were compared with ANOVA and comparisons made by Student's *t* test. Significance was defined as p value of < 0.05.

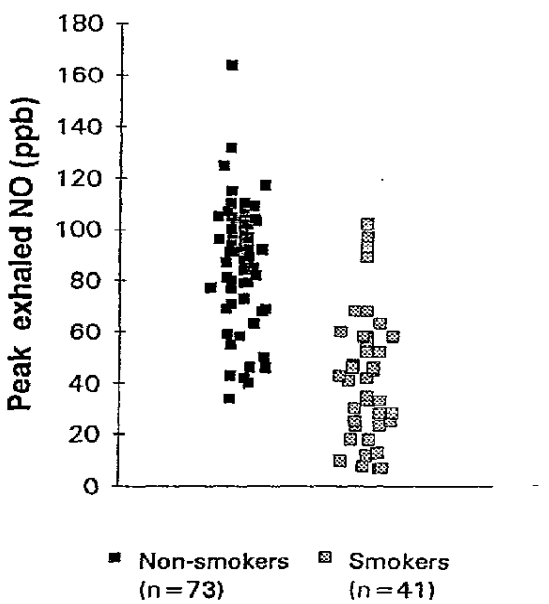

Figure 1. Peak exhaled NO levels (ppb) in nonsmokers (n = 73) and current smokers (n = 41). Exhaled NO was measured after the smokers had refrained from smoking for > 8 h ($p < 0.01$).

RESULTS

Exhaled nitric oxide in smokers. Exhaled NO was detected in all subjects. Smoking subjects had significantly decreased NO levels compared with nonsmokers (42 ± 3.9 versus 88 ± 2.7 ppb, $p < 0.01$, Figure 1). Furthermore, the amount smoked expressed as cigarettes/day was significantly correlated with the peak NO levels ($r = -0.77$, $p < 0.001$, Figure 2) and with the number of pack-years ($r = -0.67$, $p < 0.001$). Spirometry showed that all subjects had an FEV_1 within the normal range.

Acute cigarette smoke exposure caused a reduction in peak exhaled NO from 65 ± 6.3 ppb to 44 ± 6.4 ppb, which was significant, 5 min after smoking ($p < 0.02$, $n = 17$). However, NO levels returned to control values within 15 min (53 ± 6.3 ppb, $p < 0.05$).

Effects of carbon dioxide and nitric oxide. There was an increase in exhaled CO concentration from 2.0 ± 0.1 parts per million (ppm, vol/vol) to 12.0 ± 2.0 ppm ($p < 0.05$, $n = 5$) 5 min after inhalation of CO, and it remained elevated at 15 min (6.8 ± 0.62 ppm, $p < 0.05$). However, there was no change in concentration of exhaled NO (101 ± 37 ppb to 99 ± 35 ppb at 5 min, $n = 5$).

After inhalation of NO there was no significant change in peak exhaled NO concentration (122 ± 9.8 ppb before, 134 ± 10.7 ppb at 5 min, and 128 ± 15.9 ppb at 15 min after inhalation).

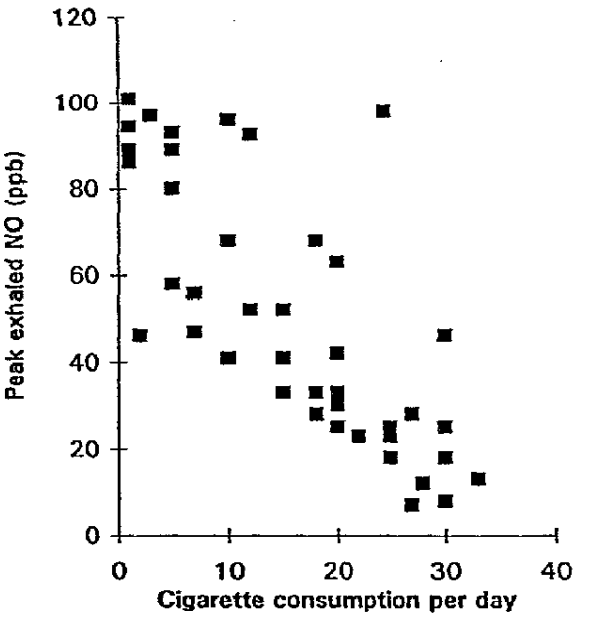

Figure 2. Linear regression between peak exhaled NO and daily consumption of cigarettes in 41 smokers. The correlation coefficient (r) was −0.77 ($p < 0.001$).

DISCUSSION

These studies demonstrate that chronic cigarette smoking is associated with a reduction in the concentration of NO in exhaled air. The exhaled NO concentration was significantly reduced in current smokers, compared with values measured in age-matched nonsmokers. Furthermore, there was a significant relationship between the daily consumption of cigarettes and the reduction in exhaled NO concentration. Acute exposure to cigarette smoke causes a small and transient reduction in exhaled NO concentration in smokers with exhaled NO values of > 20 ppb.

It is likely that NO detected in the exhaled air originates from cells lining the respiratory tract, because NO has a very short half-life (25). We have previously shown that inhalation of NO with a breath hold of 15 s causes no increase in the concentration of exhaled NO. We have also demonstrated that the NOS inhibitor N^G-monomethyl L-arginine (L-NMMA) reduces exhaled NO without any systemic effects when given by inhalation, thus suggesting inhibition of NOS within the respiratory tract (20). Recent evidence suggests that in normal subjects a large proportion of exhaled NO may be derived from the upper respiratory tract (26, 27), and therefore the effect of cigarette smoke may be exerted on cells of the nasopharynx. Indeed, the values of nasopharyngeal NO are reported to be lower in smokers than nonsmokers (26). The cellular origin of exhaled NO is uncertain, but immunocytochemical staining indicates that alveolar epithelial cells and airway epithelial cells may express constitutive NOS (14, 28). It is not known which cells in the upper respiratory tract contribute to the high levels of exhaled NO recorded in nasal samples.

The mechanism by which cigarette smoking reduces exhaled NO is not certain. Because NO itself appears to reduce the activity of NOS (15–17), the high concentration of NO in tobacco smoke may downregulate the enzyme in cells of the respiratory tract (18), resulting in a reduction in exhaled NO. We were unable to demonstrate any reduction in NO after short-term exposure to a high concentration of inhaled NO gas, although the concentration in cigarette smoke may be higher, and this does not preclude an effect with more chronic exposure. Furthermore, patients with adult respiratory distress syndrome treated with continuous inhalation of NO for up to 53 days do not appear to lose the beneficial effect on pulmonary vascular pressures (29). Another possible explanation for the reduction in exhaled NO, particularly after acute exposure, is an inhibitory effect of CO, which is also present in cigarette smoke. Carbon monoxide interacts with heme proteins, such as NOS, and potently inhibits both constitutive and inducible forms of the enzyme (24). However, exposure to a concentration of CO similar to that found in tobacco smoke failed to reduce exhaled NO concentrations in normal individuals. It is possible that bacteria in the upper respiratory tract may contribute to the higher NO values reported in the nasopharynx compared with the lower respiratory tract, because patients on antibiotics have lower values, although no controlled study has been reported (26). Cigarette smoking could therefore inhibit bacterial production of NO, or the smoke may have antimicrobial properties by virtue of its high NO content (30), although this is unlikely to account for the acute effect we have observed. In patients with bronchiectasis who have an elevated level of exhaled NO, antibiotic treatment was not associated with lower levels of exhaled NO (22). Cigarette smoke contains many different constituents, and in future studies it would be interesting to examine their effects on NOS activity. Indeed, in preliminary studies we have demonstrated that cigarette smoke extract inhibits iNOS activity and expression in cultured murine epithelial cells, apparently at the level of gene expression (R. A. Robbins, A. Robichaud, and P. J. Barnes, unpublished).

Cigarette smoking is associated with a number of adverse health effects, and several of these may be explained by a reduced production of NO. Nitric oxide production by airway epithelial cells may be important in defending the respiratory tract against inhaled infectious agents, because NO has antimicrobial properties (7, 30) and is involved in the killing of worms and parasites (8). Any reduction in epithelial NO production may therefore predispose patients to infections of the lower respiratory tract. Furthermore, endogenous NO appears to be important for the normal beating of airway epithelial cilia, which is an important mechanism for clearing debris from the respiratory tract, particularly in peripheral airways, but also in the upper respiratory tract (27, 31). Reduction in endogenous production of NO by the respiratory tract may increase the risk of infection by several mechanisms, and this may contribute to the increased risk of respiratory infection in cigarette smokers (3, 4). Nitric oxide also has an inhibitory effect on neutrophil adhesion to vessels (32) and may modulate chemotaxis of neutrophils (33). Suppression of endogenous NO may therefore predispose subjects to the neutrophilic inflammation in the respiratory tract that is associated with cigarette smoking (34).

Cigarette smoking is also associated with an increased risk of cardiovascular diseases, including atherosclerosis, peripheral vascular ischemia, and hypertension, although the mechanisms are incompletely understood (1, 4). Basal production of NO produced by eNOS activity in the systemic circulation may be an important regulator of vascular tone. Infusion of the NOS inhibitor L-NMMA in normal individuals results in an increase in blood pressure (35), indicating that basal production of endothelial NO counteracts hypertension. Furthermore, endothelium-derived NO also plays an important role in preventing platelet adhesion to endothelial cells and therefore may also counteract atherosclerosis (5). If cigarette smoking reduces endogenous NO production by inhibiting the activity of constitutive NOS, this may account for an increased risk of hypertension and peripheral vascular disease and may also account for the increased risk

of atherosclerotic disease. This possibility is supported by the recent observation that cigarette smoking is associated with a dose-related and potentially reversible impairment of endothelium-dependent dilatation in healthy young adults (36). Inhaled cigarette smoke therefore appears to be able to inhibit endogenous NO production by systemic vascular endothelium in the respiratory tract.

Nitric oxide is also released from human pulmonary endothelial cells (37), and endothelium-derived NO is important in counteracting hypoxic pulmonary vasoconstriction (38). Recent evidence suggests that L-NMMA increases pulmonary vascular resistance in normal subjects, indicating that there is a basal production of NO by pulmonary vessels (39). Cigarette smoke may have an inhibitory effect on NO release from the pulmonary vasculature and thus predispose patients to the development of pulmonary hypertension.

Our study shows that cigarette smoking reduces exhaled NO, which may reflect an inhibitory effect of cigarette smoke constituents on NOS. This effect could theoretically contribute to the increased risks of respiratory tract infections and cardiovascular disease in cigarette smokers. In the future, it will be important to identify the constituents of cigarette smoke that are responsible for this inhibitory effect.

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